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Mineral Composition through Soil-Wine System of Portuguese Vineyards and Its Potential for Wine Traceability

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Received: 29 September 2018; Accepted: 7 November 2018; Published: 9 November 2018

Abstract: The control of geographic origin is one of a highest priority issue regarding traceability and wine authenticity. The current study aimed to examine whether elemental composition can be used for the discrimination of wines according to geographical origin, taking into account the effects of soil, winemaking process, and year of production. The elemental composition of soils, grapes, musts, and wines from three DO (Designations of Origin) and for two vintage years was determined by using the ICP-MS semi-quantitative method, followed by multivariate statistical analysis. The elemental composition of soils varied according to geological formations, and for some elements, the variation due to soil provenance was also observed in musts and wines. Li, Mn, Sr and rare-earth elements (REE) allowed wine discrimination according to vineyard. Results evidenced the influence of winemaking processes and of vintage year on the wine's elemental composition. The mineral composition pattern is transferred through the soil-wine system, and differences observed for soils are reflected in grape musts and wines, but not for all elements. Results suggest that winemaking processes and vintage year should be taken into account for the use of elemental composition as a tool for wine traceability. Therefore, understanding the evolution of mineral pattern composition from soil to wine, and how it is influenced by the climatic year, is indispensable for traceability purposes.

Keywords: geographic origin; geological material; multi-element composition; rare earth elements; vinification

1. Introduction

Closely linked to the perception that terroir determines the quality and character of wines, the control of geographic origin is one of the most challenging and highest priority issues regarding traceability and wine authenticity [1]. Regional differences in sensory characteristics of wines have commonly been attributed to, among others things, (e.g., grape variety, technological processes, and vintage), local variations in soil composition [2]. Over the past two decades, many efforts have been made to identify potential fingerprints and develop reliable analytical methods to determine

the authenticity of wine [3–9]. Soil-related fingerprints justify special attention, given that there is a relationship between the chemical composition of wine and the composition of the provenance soil.

The most explored fingerprinting techniques combine chemical analysis, namely elemental and isotope ratio analysis, and multivariate statistical analysis of the chemical data to classify wines according to their geographical origin. The successful application of the techniques based on the multi-element composition of a wine strongly depends on the selection of suitable elements that would reflect the relationship with soil geochemistry, and therefore, have discriminating potential. Thus, the data on mineral elements in wine as a probe for origin determination has to be carefully interpreted, since there are many environmental, agricultural, and oenological factors that can easily mask vital elemental information [10,11]. Otherwise, wine classification will reflect not only the geographical provenance, but also anthropogenic factors.

An important source of metal content in wine comes from the vineyard soil via grapevine roots, being influenced by soil geochemistry and vine rootstock, among other factors. Other potential sources, introduced during the processing stages, from vine culture to aged wine, are atmospheric pollution, soil amendments, fertilizers, pesticides, irrigation water, contact materials during transport, vinification, and aging processes, enological processing aids, and additives [5,12–14]. Also, depletion of some elements occurs over time, especially during alcoholic fermentation. Precipitation of K and Ca as tartrate salts begins during alcoholic fermentation and continues during the aging period. The precipitation of heavy metals as insoluble salts, namely as sulfides, is favoured by sulfur dioxide addition during winemaking [2]. All these factors may markedly change the multi-element composition of the wine, affecting the relationship between wine and soil compositions, thus precluding their use for authentication purposes.

Alkaline elements, Li, Rb, and Cs, are good indicators of geographical origin, as they are not included in the group of contaminant elements of wines [10]. Mn, Mg, Sr, Ba, and rare earth elements (REE) are also listed as useful elements, although the first two should be considered carefully, as they can be introduced through viticultural practices such as the use of fertilizers and pesticides, while REE content in wines can increase due to treatment with bentonites [11].

Studies on this subject have been pursued in most wine producing-countries, such as Argentina [15], Australia [7,16], Brazil [17], Canada [3,18], Germany [5], Italy [19,20], Portugal [4,21], Romania [22,23], South Africa [8,24,25], and Spain [26,27], indicating that the multi-elemental determination of wines can enable their successful discrimination. Most of the aforementioned studies are exclusively focused on wines produced under controlled conditions in order to guarantee their authenticity. Studies, involving wines and the provenance soils, are scarce [3,4,22].

The simultaneous use of a greater number of variables can provide increasingly robust results for the identification of wine geographical origin. Some studies based on this approach are described, with the most chosen variables being multi-element composition, strontium isotopic ratio ($^{87}Sr/^{86}Sr$), and stable isotope ratios of light elements [6,28]. Within a research program regarding strategies for wine fingerprinting, REE and $^{87}Sr/^{86}Sr$ were identified as viable tools for traceability of Portuguese DO in wines, where soils are developed on different geological formations [6,9], and studies on their robustness are underway [29,30].

Portugal is the eleventh largest producer of wine and the nine largest exporter in the world, with over six hundred millions of liters produced in 2016 [31]. Nevertheless, there is little information on multi-elemental analysis of Portuguese wines for their classification according to geographical origin. In this context, a study was developed to investigate whether inter-regional variation in multi-element composition could be used as a tool for the traceability of three Portuguese Designations of Origin (DO), where soils are developed on different geological formations. Having in mind the natural heterogeneity of some wine regions in terms of geological materials and soils, intra-regional variability was also studied by involving different vineyards from the same DO. Also, the evolution of mineral composition during vinification and inter-year variability were considered. Results will enlarge global databanks on wine composition and support comparisons with other world regions.

2. Materials and Methods

2.1. Vineyards

Four vineyards from three Portuguese DO (Dão, Óbidos and Palmela) were studied. In the Palmela DO (Southern Portugal), one vineyard of José Maria da Fonseca was considered: Vinha de Algeruz (AL; 38°34′ N, 8°49′ W), established on Haplic Eutric Arenosols, and Regosols, developed on Pliocene sedimentary formations (sands with clay beds), the most representative geological formation of Palmela DO. In the Óbidos DO (Centre of Portugal), two vineyards belonging to Companhia Agrícola do Sanguinhal were considered: Quinta de S. Francisco (SF; 39°11′ N, 9°10′ W) and Quinta do Sanguinhal (SA; 39°15′ N, 9°09′ W); the former is established on Eutric Regosols which developed on Lower Cretaceous sandstones, and the latter on Dystric Regosols, developed on Upper Jurassic clayey sandstones. In the Dão DO (Central Portugal), one vineyard of Sogrape Vinhos was considered: Quinta dos Carvalhais (QC; 40°33′ N, 7°47′ W), established on Dystric Cambisols, and Dystric Regosols, developed on monzonitic granites (310–350 MY), the most representative geological formation of Dão DO. The area of each vineyard, year of planting, rootstock, vine spacing, row orientation, and training system are indicated in the Table 1. All the vineyards had the same red variety in production (*Vitis vinifera* L., cv Aragonez).

The study areas have a Mediterranean-type climate. The QC, SA, and SF vineyards have a climate with dry summers and cold nights (Csb, Köppen classification), whereas the AL vineyard has a climate with dry hot summers and temperate nights (Csa, Köppen classification) [32]. Annual rainfall is variable, and in 2010, was much higher than in 2009 (for example 1598 versus 953 mm and 760 versus 1006 mm in Óbidos and Palmela DOs, respectively [33]. Soils of each vineyard show specific particle-size distribution which is associated with the respective geological formations, and their main characteristics are uniform. In the five years prior to this study, several treatment products (namely pesticides and fertilizers) were used, and may have contributed to B, Mg, Al, K, Ca, Cu, and Zn soil enrichments.

Table 1. Vineyards characteristics. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (QC) Quinta dos Carvalhais.

Vineyard/ Portuguese DO	Area of the Vineyard (ha)	Year of Planting	Rootstock	Vine Spacing (m)	Row Orientation	Training System
AL vineyard/Palmela	3.0	1990	1103P	2.8 × 1.2	N-S	bi-lateral cordon
SF vineyard/Óbidos	5.0	2001	R110	2.7 × 1.0	N-S and E-W	bi-lateral cordon
SA vineyard/Óbidos	2.6	2000	R110	2.7 × 1.0	N-S	bi-lateral cordon
QC vineyard/Dão	2.5	1995	1103P	2.0 × 1.2	NE-SW	bi-lateral cordon

2.2. Soil and Grape Berries Sampling

Soil sampling took place in December 2007 (AL, SF, SA) and May 2009 (QC). Soil samples were collected with an auger from nine sampling sites distributed along three non-contiguous vine rows (representative of the entire vineyard area), considering both row and inter-row. For evaluation of soil composition heterogeneity with depth, samples were taken from five depth layers (0–20, 20–40, 40–60, 60–80 and 80–100 cm), and sealed in plastic bags (Figure 1). The samples used in the present study were those collected at the 0–20, 40–60 and 60–80 cm layers after mixing the respective row and inter-row samples.

Grape berries were sampled two weeks before the 2009 harvest. A total of 500 grape berries were collected, using plastic gloves, from the four grapevines around each soil sampling point, stored in plastic bags, and kept at cool temperatures until their processing.

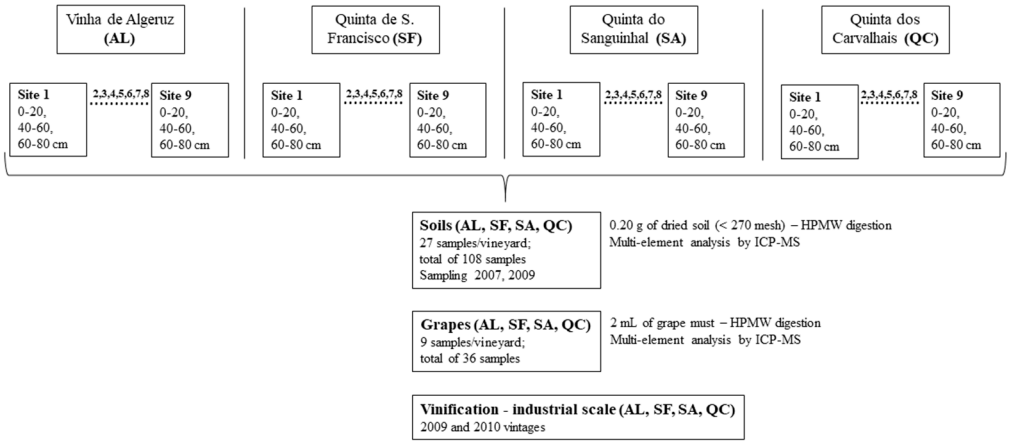


Figure 1. Schematics of the sampling strategy and analytical procedures used in this study.

2.3. Winemaking and Wine Sampling

For each vineyard, wine was prepared using all the grapes grown in the respective selected area in two consecutive harvests, 2009 and 2010. The wines were produced at three different wineries (corresponding to the wine companies participating in this study), at an industrial scale, following the traditional red winemaking process (Figure 2), with slight technological differences between wineries.

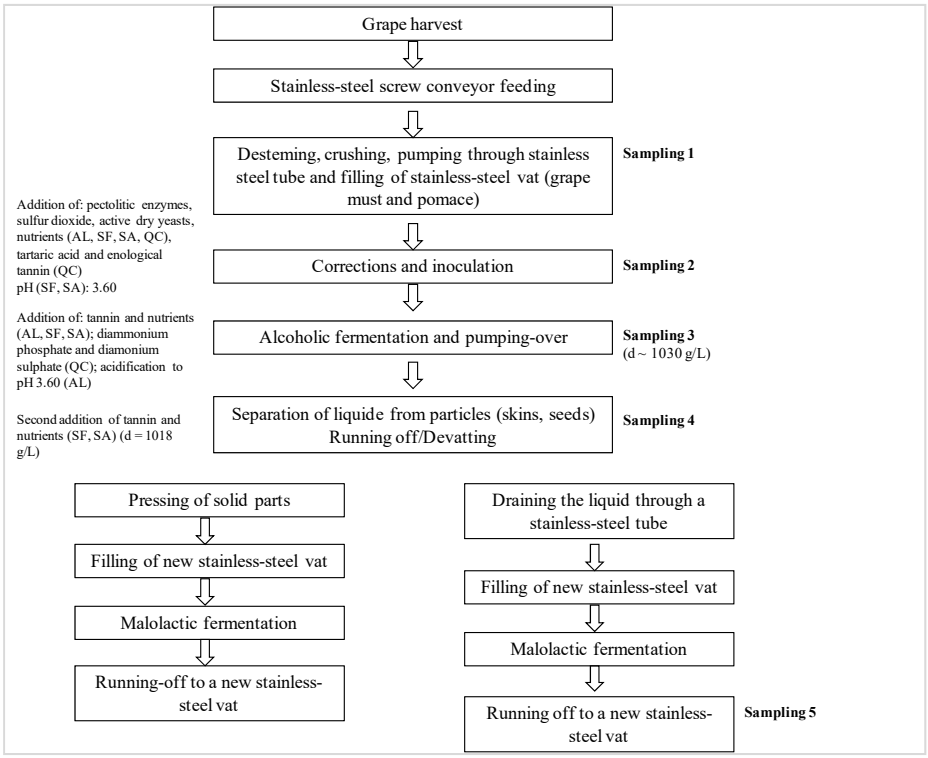


Figure 2. Schematics of the winemaking processes with indication of the points where samples were collected. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (QC) Quinta dos Carvalhais.

In the 2009 vintage, samples were collected in decontaminated polyethylene tubes, in triplicate, at five different steps of the winemaking process (2009 vintage): (1) after crushing and vatting; (2) after must corrections and yeast inoculation; (3) during the alcoholic fermentation (density approximately of

1030 g/L); (4) after running off; (5) after malolactic fermentation and first racking, before any blending to preserve the trace to the vineyard of origin. Sampling 5 was repeated for the 2010 vintage.

Immediately after sampling, a volume of 5 mL of ultrapure HNO_3 was added to a sample volume of 45 mL, in order to stop the alcoholic fermentation and to start organic matter decomposition.

2.4. Sample Processing

For ICP-MS analysis, soils, grape musts and wines were treated by high pressure microwave digestion (HPMW). Soil samples were dried at room temperature, ground, and forced to pass through a sieve of 2 mm. A subsample of 0.20 g of dried soil (fraction < 0.053 mm) was acid digested using a microwave system as described by Martins et al. [9]. A certified reference material (Geo PT 25, Basalt HTB-1, International Association of Geoanalysts) was used as a reference for quality control of the analytical results.

Grape must was prepared in the laboratory by smashing the berries in plastic cups and then transferring the juice into polyethylene flasks. Immediately thereafter, a volume of 5 mL of ultrapure HNO_3 was added to a sample volume of 100 mL. Both grape musts and wines were acid digested following a digestion program previously optimized by Catarino et al. [34]. A Milestone ETHOS Plus Microwave Labstation (Milestone, Sorisole, Italy), equipped with a Milestone HPR-1000/6m monoblock high pressure rotor and TFM Teflon vessels, was used.

To avoid contamination, all polyethylene material (volumetric flasks, micropipette tips, and autosampler vessels) was immersed at least for 24 h in 20% (v/v) HNO_3, and rinsed thoroughly with purified water before use. For decontamination solution preparation, reagent grade HNO_3 was double-distilled using an infra-red subboiling distillatory system (model BSB-939-IR, Berghof, Germany). Purified water (conductivity < 0.1 μS cm^{-1}) was produced using a Seralpur Pro 90CN apparatus (Seral, Ransbach-Baumbach, Germany).

2.5. Multi-Elemental Analysis

Multi-elemental analysis was carried out with anElan 9000 ICP-MS (Perkin-Elmer SCIEX, Norwalk, CT, USA) equipped with a cross-flow nebulizer, a Ryton Scott-type spray chamber, and nickel cones. A four-channel peristaltic sample delivery pump (Gilson model) and a Perkin-Elmer AS-93 Plus autosampler (Perkin-Elmer SCIEX, Norwalk, CT, USA) protected by a laminar-flow-chamber clean room class 100 (Max Petek Reinraumtechnik, Radolfzell am Bodensee, Germany) were used. The ICP-MS instrument was controlled by Elan 6100 Windows NT software (Version 2.4, Perkin-Elmer SCIEX, Norwalk, CT, USA). The operating conditions of the ICP-MS equipment were as follows: radio-frequency (RF) power of 1200 W; Ar gas flow rates of 15 L/min for cooling, between 0.94 and 0.98 L/min for nebulizer and 1.5 L/min for auxiliary; and solution uptake rate of 1.0 mL/min.

Element concentrations were determined in mineralized soil samples (after 100-fold dilution) and in mineralized musts and wines (after 10-fold dilution), in duplicate, by adapting the ICP-MS semi-quantitative method previously described by Catarino et al. [35]. A full mass spectrum (m/z = 6–240, omitting the mass ranges 16–18; 40, 41, 211–229) was obtained by full mass range scanning. Rh and Re (10 μg/L) were used as internal standards.

The reference response table (Perkin-Elmer TotalQuant III, Perkin-Elmer SCIEX, Norwalk, CT, USA) was updated with different multi-elemental standard solutions with appropriate concentrations for soil analyses, and for must and wine analyses. A certified reference material (GeoPT 25, Basalt HTB-1) was periodically analysed for quality control. Also, for quality control purposes, wines from an intercomparison OIV (International Organisation of Vine and Wine) trial were analysed periodically. Between determinations, the equipment sampling system was rinsed with a 2% HNO_3 (v/v) for 75 s.

2.6. Statistical Analysis

Statistical analysis of the multi-element data was carried out to evaluate: (1) the effect of the vineyard of origin (Vineyard) on soil mineral composition; (2) the effect of soil depth level on soil

mineral composition; (3) the effect of Vineyard on mineral composition of grape musts (prepared at laboratory from the grape berries sampled in the vineyards); (4) the effect of winemaking stage (Vinification) on mineral composition of wines; (5) the effect of the Vineyard on the mineral composition of wines. The statistical analysis was firstly performed by one-way analysis of variance and comparison of means (Fisher LSD, 95% level) using Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA). Normal distribution and homogeneity of variance were verified by Normal p-p (distribution of within-cell residuals) and Cochran C tests ($p < 0.05$), respectively. Whenever the parametric test assumptions were not verified, a non-parametric test (Kruskal-Wallis test) was applied. In these cases, comparison of means was not carried out. Multivariate statistical analysis, principal component analysis (PCA), and discriminant analysis (DA), was then performed using NTSYS-pc package software (Version 2.1q, Exeter Software, Setauket, NY, USA) [36] and Statistica 7.0 software (TIBCO Software Inc., Palo Alto, CA, USA), respectively. For each matrix type (soils, grape musts and wines), the results were submitted to aggregation analysis and PCA. Finally, DA was applied to the data, considering Li, Mn, Sr, and REE as variables, and the vineyards as groups.

3. Results and Discussion

3.1. Mineral Composition of Vineyard Soils

Results of the multi-element analysis and variance analysis (one-way ANOVA) of the soils of the different vineyards are listed in Table 2. It is worth mentioning that the total concentrations of the elements observed in the soils of the four vineyards are compatible with the contents of uncontaminated soils [37]. A significant effect of Vineyard on soil composition was observed for all the studied elements. As a general trend, the soil from QC (Dão DO), developed on granites, showed higher concentrations than soils from the other vineyards developed on geological sedimentary formations. This trend was especially noticeable for Li, Be, Al, Fe, Ga, Ge, As, Rb, Sn, Cs, and REE. The soil from the vineyard of Palmela DO, developed on Pliocenic sandy materials, showed the lowest concentrations for most of the elements, with Na being an exception. Data for some elements (e.g., Li, Be, Rb, Cs) in the studied vineyards are different from those reported by Almeida and Vasconcelos [4] for two vineyards from DO Douro region, installed in soils developed on schists.

Despite differences in age of the geological formations, the two vineyards from Óbidos DO showed similar concentrations for some elements, which may be explained by similar characteristics of their soils (Table S1).

Different patterns were observed in the studied vineyards (Table S2) concerning the effect of soil depth on element concentration. In fact, in soils from Palmela DO, for most of the elements, a decrease in concentration was observed with increasing depth. In contrast, for QC soil (Dão DO), no significant effect of soil depth was observed. Such a difference may be associated with the degree of soil disturbance for vineyard installation, with stronger and deeper soil disturbance occurring when a bulldozer has homogenized the different soil layers up to 100 cm depth.

Content patterns observed for Li, Be, Mn, As, Rb, Sr, and Cs suggest that these elements might be potential discriminant elements, allowing soil differentiation between the studied DOs. Indeed, similar concentrations for these elements were observed in the soils from Óbidos DO vineyards, while different concentrations were found in those from other DOs, in particular in the soil from Dão DO.

It should be emphasized that only a small proportion of total metal concentration is potentially extractable by plants, as elements are mostly strongly bonded within mineral structures. In addition, the ecotoxicology and mobility of metals in the soil depend strongly on specific chemical forms in which they are present [38].

Table 2. Multi-elemental composition (μg/g) of soils of the different vineyards. Average concentration (mean ± standard deviation). (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (CQ) Quinta dos Carvalhais.

Element	Vineyard Effect	AL Vineyard /Palmela	SF Vineyard /Óbidos	SA Vineyard /Óbidos	QC Vineyard /Dão
Li	**	23 ± 6	33 ± 7	39 ± 7	224 ± 50
Be	**	1.1 ± 0.3	2 ± 1	2.0 ± 0.4	31 ± 10
Na	**	4043 ± 1134	1555 ± 317	2561 ± 471	2570 ± 1081
Mg	**	737 ± 214	3226 ± 1839	1612 ± 430	5690 ± 898
Al	**	41,987 ± 9113	76,330 ± 19,270	49,982 ± 6832	149,501 ± 14,233
Ca	**	1759 ± 427	1775 ± 970	1046 ± 513	1850 ± 1126
Ti	**	5 ± 2	10 ± 3	8 ± 2	9 ± 1
V	**	20 ± 6	75 ± 29	40 ± 9	69 ± 12
Cr	**	17 ± 4	63 ± 27	29 ± 7	52 ± 12
Mn	**	87 ± 24	1147 ± 662	1166 ± 477	514 ± 105
Fe	**	6530 ± 2060	27,390 ± 11,737	12,580 ± 2877	40,450 ± 5543
Co	**	2 ± 1	15 ± 7	8 ± 2	9 ± 2
Cu	*	82 ± 39	87 ± 29	117 ± 61	79 ± 30
Ga	**	9 ± 2	19 ± 5	13 ± 2	38 ± 4
Ge	**	0.26 ± 0.06	0.31 ± 0.05	0.29 ± 0.05	0.52 ± 0.07
As	**	6 ± 3	11 ± 4	9 ± 3	46 ± 12
Rb	**	165 ± 43	190 ± 23	188 ± 27	333 ± 52
Sr	**	59 ± 15	57 ± 6	57 ± 17	34 ± 4
Sn	**	7 ± 3	6 ± 1	6 ± 2	25 ± 3
Sb	**	1.4 ± 0.9	0.8 ± 0.2	0.8 ± 0.3	0.38 ± 0.07
I	**	0.2 ± 0.1	0.6 ± 0.4	0.5 ± 0.4	0.5 ± 0.2
Cs	**	5 ± 1	9 ± 2	8 ± 1	55 ± 10
Ba	**	663 ± 316	591 ± 375	501 ± 57	305 ± 41
La	*	19 ± 5	22 ± 8	16 ± 7	23 ± 11
Ce	**	44 ± 11	49 ± 19	38 ± 15	59 ± 26
Pr	*	5 ± 1	6 ± 2	5 ± 2	7 ± 3
Nd	**	20 ± 4	22 ± 8	19 ± 7	28 ± 13
Sm	**	3.7 ± 0.9	5 ± 1	4 ± 1	6 ± 2
Eu	**	0.6 ± 0.1	1.0 ± 0.3	0.7 ± 0.1	1.0 ± 0.3
Gd	**	3 ± 1	4 ± 1	4 ± 1	6 ± 2
Tb	**	0.36 ± 0.08	0.6 ± 0.2	0.5 ± 0.1	0.9 ± 0.3
Dy	**	1.8 ± 0.4	3 ± 1	2.6 ± 0.6	5 ± 2
Ho	**	0.31 ± 0.08	0.6 ± 0.2	0.5 ± 0.1	0.9 ± 0.3
Er	**	0.9 ± 0.2	1.9 ± 0.6	1.4 ± 0.3	2.5 ± 0.8
Tm	**	0.13 ± 0.04	0.27 ± 0.08	0.21 ± 0.04	0.4 ± 0.1
Yb	**	1.0 ± 0.3	1.8 ± 0.6	1.5 ± 0.3	2.4 ± 0.6
Lu	**	0.14 ± 0.04	0.27 ± 0.08	0.23 ± 0.04	0.35 ± 0.08
W	**	0.004 ± 0.001	0.007 ± 0.002	0.006 ± 0.001	0.045 ± 0.015
Tl	**	0.9 ± 0.3	1.1 ± 0.1	1.3 ± 0.2	2.4 ± 0.3

* Significant effect ($p < 0.05$); ** significant effect ($p < 0.01$). For each vineyard and element, the results are based on average values of nine sampling sites and three depth levels (0–20 cm, 40–60 cm and 60–80 cm), in a total of 27 samples, analysed in duplicate.

For those elements significantly that were affected by Vineyard, a PCA was performed (Figure 3). The first two principal components explained 85% of the total variance. The variables which most influence the first component (C1, 70%) were Li, Be, Mg, Al, Fe, Ga, Ge, Rb, Cs, and several REE, and the variables which most influence the second component (C2, 15%) were Na and Mn. Using the first two principal components, the soils seems to separate according to the DO. The QC soils samples are well separated from the other, as well as the AL soils. Regarding the SA and SF soils, they presented a strong variability.

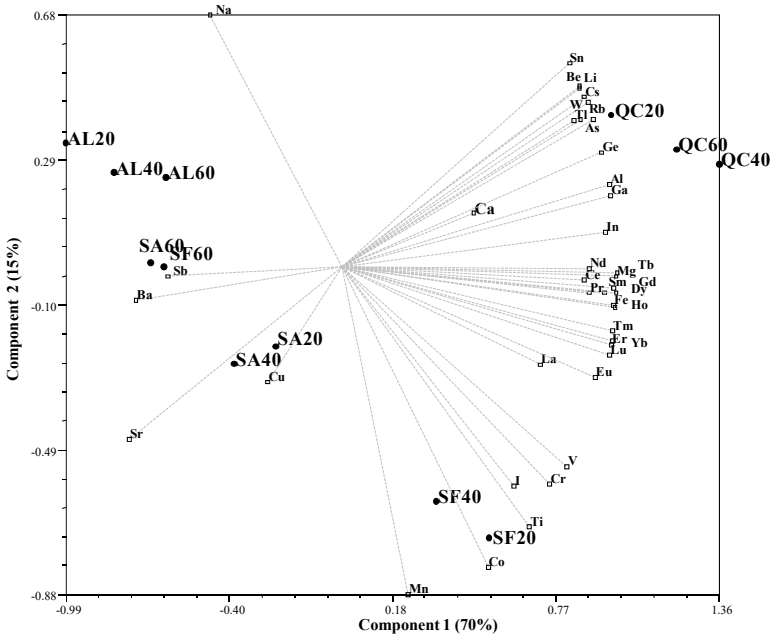


Figure 3. Principal Component Analysis performed on soils from the vineyards/DO: (AL) Vinha de Algeruz/Palmela, (SF) Quinta de S. Francisco/Óbidos, (SA) Quinta do Sanguinhal/Óbidos, and (QC) Quinta dos Carvalhais/Dão, collected at nine sampling sites and three depth levels (0–20 cm, 40–60 cm, and 60–80 cm), in a total of 27 samples, analysed in duplicate The soils are represented in the plane of the two first components which express, respectively, 70% and 15% of the total variance.

3.2. Mineral Elements Concentration during Vinification Processes

A Vineyard effect was also observed on grape must composition (data not shown, see Table S3). In fact, for most of the elements, significant differences were found between musts from different vineyards. As observed for soils, the grape must from QC showed the highest concentrations of Rb and Cs. This trend is consistent with the relationship between soil and must composition, and agrees with results reported for vineyards of Douro DO, installed in soils developed on schists [4]. However, an inverse position was observed between Óbidos and Palmela DOs, as must from AL vineyard contained higher element concentrations than those from Óbidos DO, which does not follow the trend found for the respective soils.

Several factors can modify the multi-elemental composition of grapes and wines, such as winemaking practices [10,11,13,29,30,39,40], which influence the extraction extent of mineral elements from the different parts of grape berry, element depletion by precipitation and co-precipitation phenomena, and incorporations by enological additives, among other factors. The evolution of the concentrations of elements during vinification (2009 vintage) is displayed for each vineyard (Figures 4–6), considering the following sampling steps: (1) after crushing and vatting; (2) after must corrections and inoculation; (3) during alcoholic fermentation; (4) after running off; and (5) after malolactic fermentation and first racking.

3.2.1. Alkaline and Alkaline Earth Elements

Regarding alkaline and alkaline earth elements (Figure 4), namely Li, Na, Mg, Ca, Rb, Sr, and Cs, a significant effect of Vinification was observed for all the vineyards with the exceptions of Mg, Rb, Cs, and Ba (QC). Different trends and distinct magnitudes of change were observed, depending on the element, its initial concentration, products applied and moment of application, and most probably on must-wine characteristics (pH and alcoholic strength).

With respect to Li, significant decreases occurred after corrections of the grape musts from all studied vineyards, with the greatest change (about 60%) occurring in the must/wine from the AL

vineyard. These results do not agree with those from a study involving red wines, obtained by maceration of solid parts of berries where Li enrichments were likewise observed [40]. Nevertheless, the trend loss of Li during winemaking was reported by Gómez et al. [39] for white wines.

Different trends were observed between vineyards regarding Na, but without technological relevance. The increase of Na that was verified after must corrections might be explained by the addition of sodium metabisulfite.

Despite the significant effect of Vinification on Mg concentration, the small differences observed suggest its stability over time, as Mg salts are soluble, following trends reported by [39].

Concentrations of Ca tended to decrease during vinification (with exception of SF vineyard), which is associated with the low solubility of calcium tartrate, as a natural phenomenon of physical-chemical stability. The decrease (from 99 to 72 mg/L) was more evident in the must/wine from the AL vineyard, where the highest initial Ca concentration was found. The correction of pH to 3.60 with tartaric acid, performed when alcoholic fermentation was running, might be affected by Ca concentration, as the insolubility of tartaric acid salts (potassium hydrogen tartrate and calcium tartrate) is enhanced by the presence of ethanol [2].

Concentrations of Rb slightly increased during the initial phases of vinification for SF, SA, and QC musts, probably due to extraction from solid parts of the berries, favoured by maceration during alcoholic fermentation [39]. Enrichments of Sr were noticed during alcoholic fermentation as a result of its extraction from solid parts of grape berries, mostly from seeds, for musts of the SF, SA, and QC vineyards, agreeing with observations of Catarino et al. [40]. A steady concentration was observed for Cs throughout the vinification period, while Ba and especially Sr showed increasing concentrations (with the exception of AL vineyard).

Our results indicate that for most of the elements, the evolution from must to wine regarding the AL vineyard diverged from the other grape musts. In fact, strong losses were observed for Na, Mg, Rb, Sr, and Ba during the first half of alcoholic fermentation. Possibly, the increasing concentration of ethanol promotes the precipitation or co-precipitation of these metals in wine [10]. During the second phase of the alcoholic fermentation, after acid addition for pH correction, enrichments were perceived, possibly due to metal extraction from solid parts and diffusion to the must, which are favoured by the pH decrease and by the increase of ethanol content.

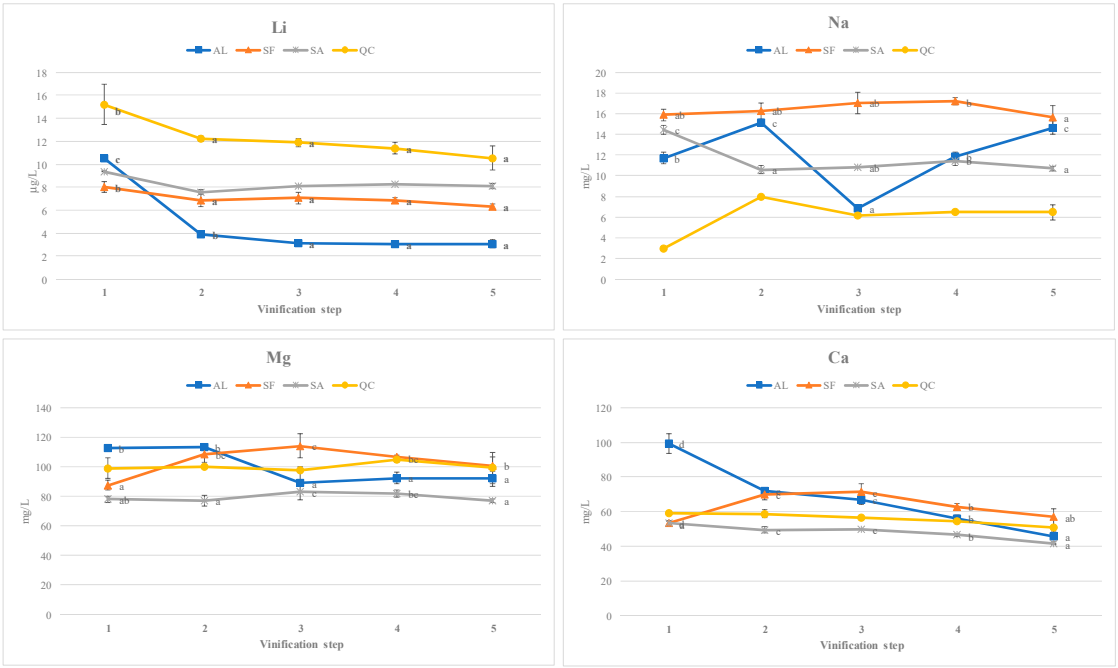


Figure 4. *Cont.*

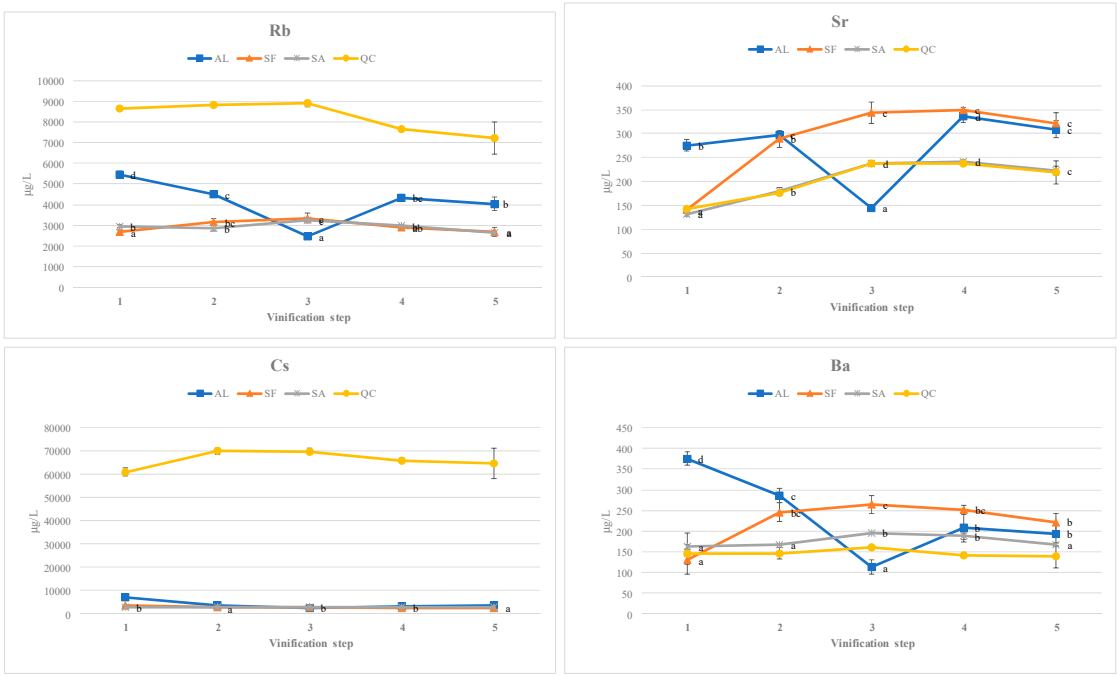


Figure 4. Evolution of alkaline and alkaline earth metals concentrations over vinification, by vineyard of origin. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (QC) Quinta dos Carvalhais. Vinification step: (1) after crushing and vatting; (2) after must corrections and inoculation; (3) during the alcoholic fermentation (density approximately of 1030 g/L); (4) after running off; (5) after malolactic fermentation and first racking, before any blending to preserve the trace to the vineyard of origin. For each element and vineyard, results correspond to mean values (and corresponding standard deviations) of three replicates and corresponding analytical duplicates (n = 6). Means followed by the same letter are not significantly different at 0.05 level of significance. Whenever values are not followed by significance letters it means that a non-parametric parametric test (Kruskal-Wallis test) was applied and comparison of means was not carried out.

Element concentrations throughout vinification suggest the importance of their distribution in grape berries, which may be strongly influenced by technology promoting the maceration of skins and seeds. In a study focusing on the Chardonnay variety, it was observed that Ca, Sr, and Ba elements accumulate mainly in the seeds, while Li, Mg, Na, Rb, and Cs accumulate mainly in the flesh [41]. Analysing our results, the late enrichments in Sr and Ba seem consistent with kinetics extraction from seeds.

Alkaline and alkaline earth metals, namely Li, Rb, and Cs, and to a low extent Mg, Sr, and Ba, are promisor indicators of geographic origin, because they can easily be absorbed by plants in the soil. Although Mg should be considered with caution as a fingerprint, because it can be associated with correctives and soil fertilizers, it should be emphasized that wines produced in southern Portuguese soils that are rich in Mg and Na [42] showed higher concentrations of these elements as compared to other wines [21]. The higher concentrations of Li and especially of Rb and Cs in must/wine from the Dão DO compared with the other DOs suggest that these elements could be promisor fingerprints for separate Portuguese DOs.

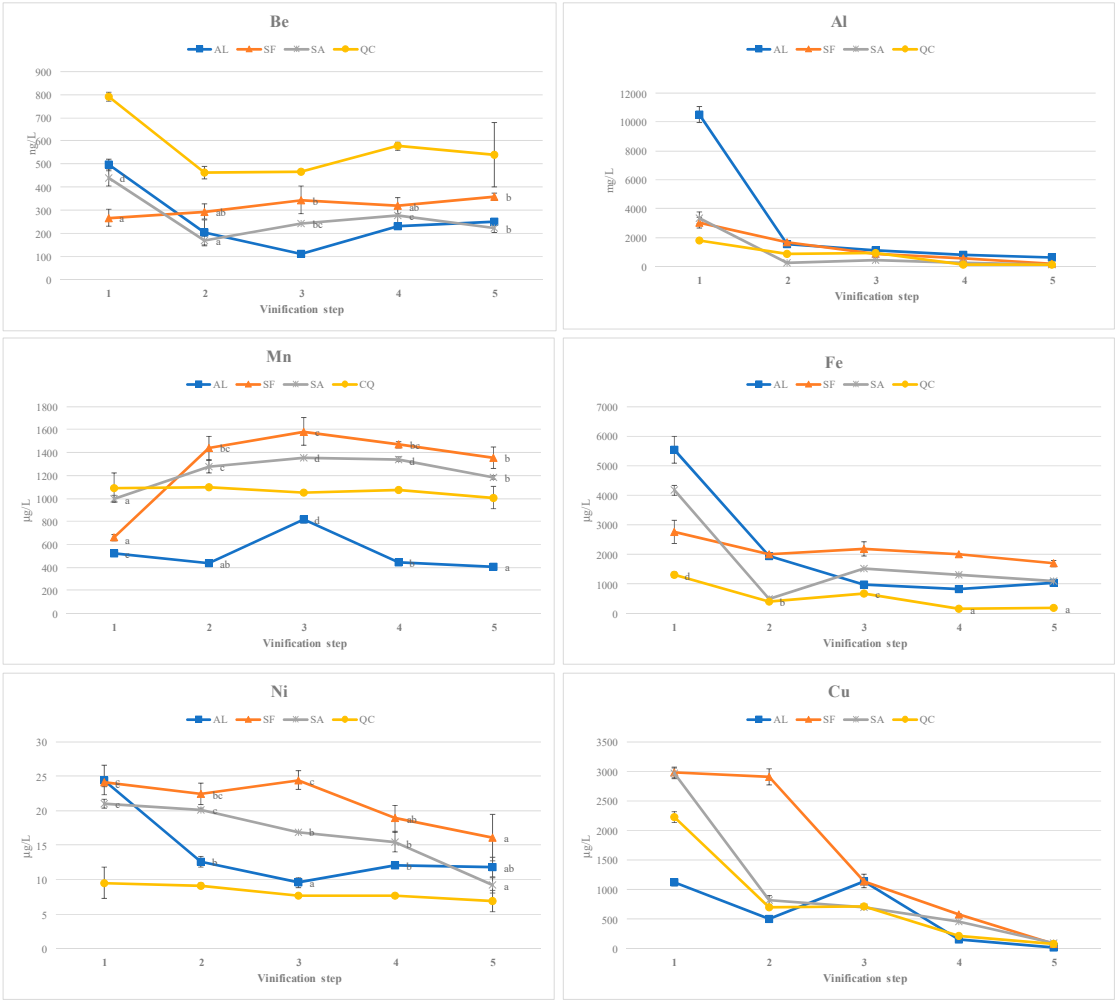
3.2.2. Contaminant Elements

Concentrations of contaminant metals, specifically Be, Al, Mn, Fe, Ni, Cu, Zn, Ga, Mo, Sn, Sb and Tl, most of them heavy metals, are shown in Figure 5. A significant effect of Vinification was observed for all these elements and vineyards, with Zn (SF and QC), Be, Mn, and Ni (QC) being exceptions. With the exception of Mn, the concentration of contaminant elements strongly decreased

during alcoholic fermentation, favoured by sulfur dioxide addition to the must, in accordance with the precipitation phenomena of heavy metals as insoluble salts, namely as sulfides, over time, as previously reported in [4,5,40].

The grape must from the vineyard AL showed for several contaminant metals, namely Al, Fe, Ni, Zn, Ga, Mo, Sb, and Tl, at the highest concentrations at step 1 of vinification, which is consistent with the results for the musts prepared at laboratory. However, no direct correspondence between must contents and final wine contents was observed, as impressive losses occurred after sulfur dioxide addition and until the moment of acidification, corroborating the important role played by sulfur dioxide as a purifying agent. In this must, depletions from step 1 to step 3 were as follows: Be-78%, Al-90%; Fe-82%; Ni-61%; Zn-75%; Ga-69%; Mo-47%; Sn-61%; Sb-78%, and Tl-71%. The depletion of these metals is positive from the wine quality perspective, given their potential participation in physical-chemical instability phenomena and potential toxicity, as excessive concentrations of Fe and Cu can cause turbidity known as ferric casse and cupric casse, respectively [2].

In contrast, slight enrichments of Be occurred during the second phase of vinification in all the musts. Surprising increases in Mn concentrations were verified in both musts from Óbidos DO after the initial corrections, suggesting contamination, which is not in agreement with a study where important losses of this metal from must to wine (red winemaking) were reported [40]. A final increase of Zn concentration in the must from AL is noteworthy; this may be of exogenous origin. In these circumstances, it is clear that contaminant elements should not be included in the group of potential markers for geographical origin fingerprints.



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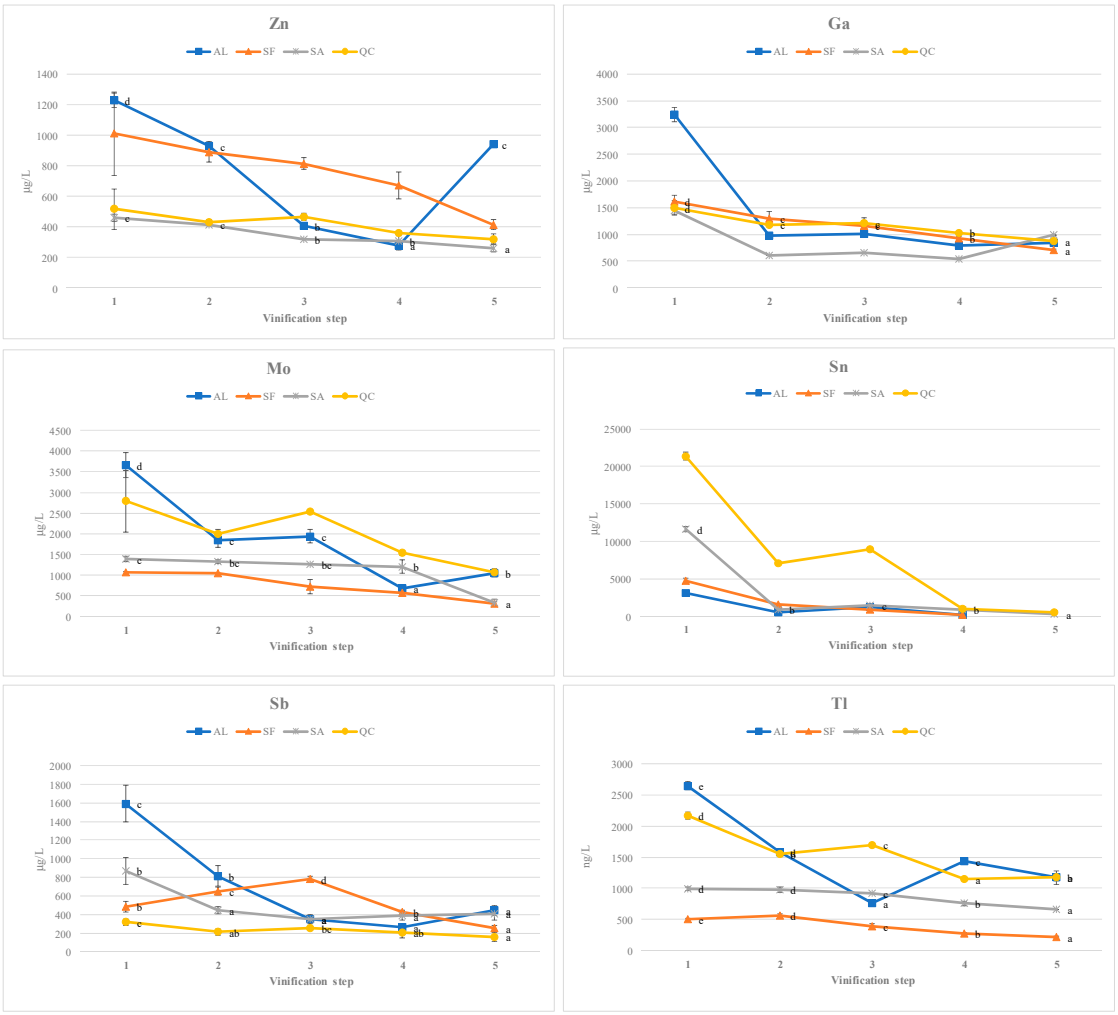


Figure 5. Evolution of heavy metals (contaminant elements) concentrations over vinification, by vineyard of origin. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (QC) Quinta dos Carvalhais. Vinification step: (1) after crushing and vatting; (2) after must corrections and inoculation; (3) during the alcoholic fermentation (density approximately of 1030 g/L); (4) after running off; (5) after malolactic fermentation and first racking, before any blending to preserve the trace to the vineyard of origin. For each element and vineyard, results correspond to mean values (and corresponding standard deviations) of three replicates and corresponding analytical duplicates ($n = 6$). Means followed by the same letter are not significantly different at 0.05 level of significance. Values are not followed by significance letters indicate that a non-parametric parametric test (Kruskal-Wallis test) was applied and comparison of means was not carried out.

3.2.3. Rare Earth Elements

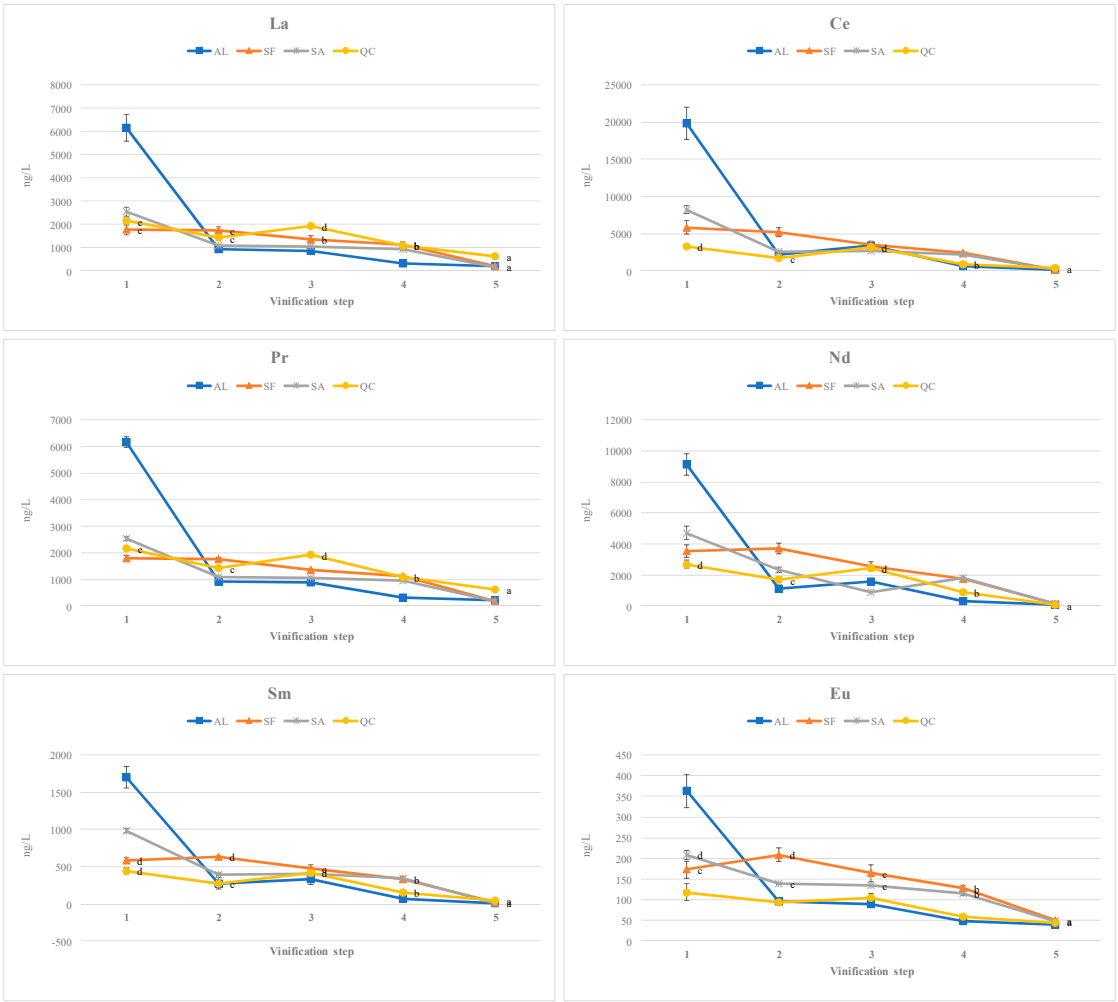
REE present particular interest for wine fingerprinting because, owing to their chemical similarity, the problem of selective changes of their concentration distribution is avoided. It seems that plants generally absorb REE from soil without any selectivity [43]. Furthermore, due to their chemical similarity, all the REE are expected to be affected to the same extent by insolubility and precipitation phenomena.

The present study allowed the understanding of the evolution of rare earth elements along several steps of vinification. Significant effect of Vinification on REE concentrations of the musts from the four vineyards was verified (Figure 6). As expected, for each must, a common trend was observed for all REE. In musts from the vineyards SF, SA, and QC, slight decreases occurred during vinification. In some cases, negligible increases during alcoholic fermentation could be related to REE preferential accumulation in the skin of berry [41].

The very high concentrations of REE in the must from the vineyard AL are notable in comparison with the others. In fact, the REE concentrations observed in the grape must, immediately after crushing and vatting, are in accordance with the concentrations found in grape berries (data not shown, see Table S3). Impressive decreases from step 1 to step 2 of vinification, in general higher than 70%, are most probably due to precipitation phenomena, favoured by high pH value of the musts.

It should be pointed out that no close relationship was observed between mineral content of soils and mineral content of grape berries and must at the beginning of vinification. In general, the highest concentrations were measured in the must from vineyard AL, despite the low concentrations in the corresponding soil. These results suggest that absorption of mineral elements in vineyard AL was easier than in the other vineyards, which may be facilitated by the low contents of clay and iron and aluminium compounds in the respective soil leading to lower interaction with the soil solution and facilitate element absorption.

Although the REE have been used mostly combined with other trace elements for wine fingerprinting, an excellent way to compare the REEs concentration ratios for soils, musts, and wines is by plotting REE concentrations on a chondrite-normalised diagram [6,44].



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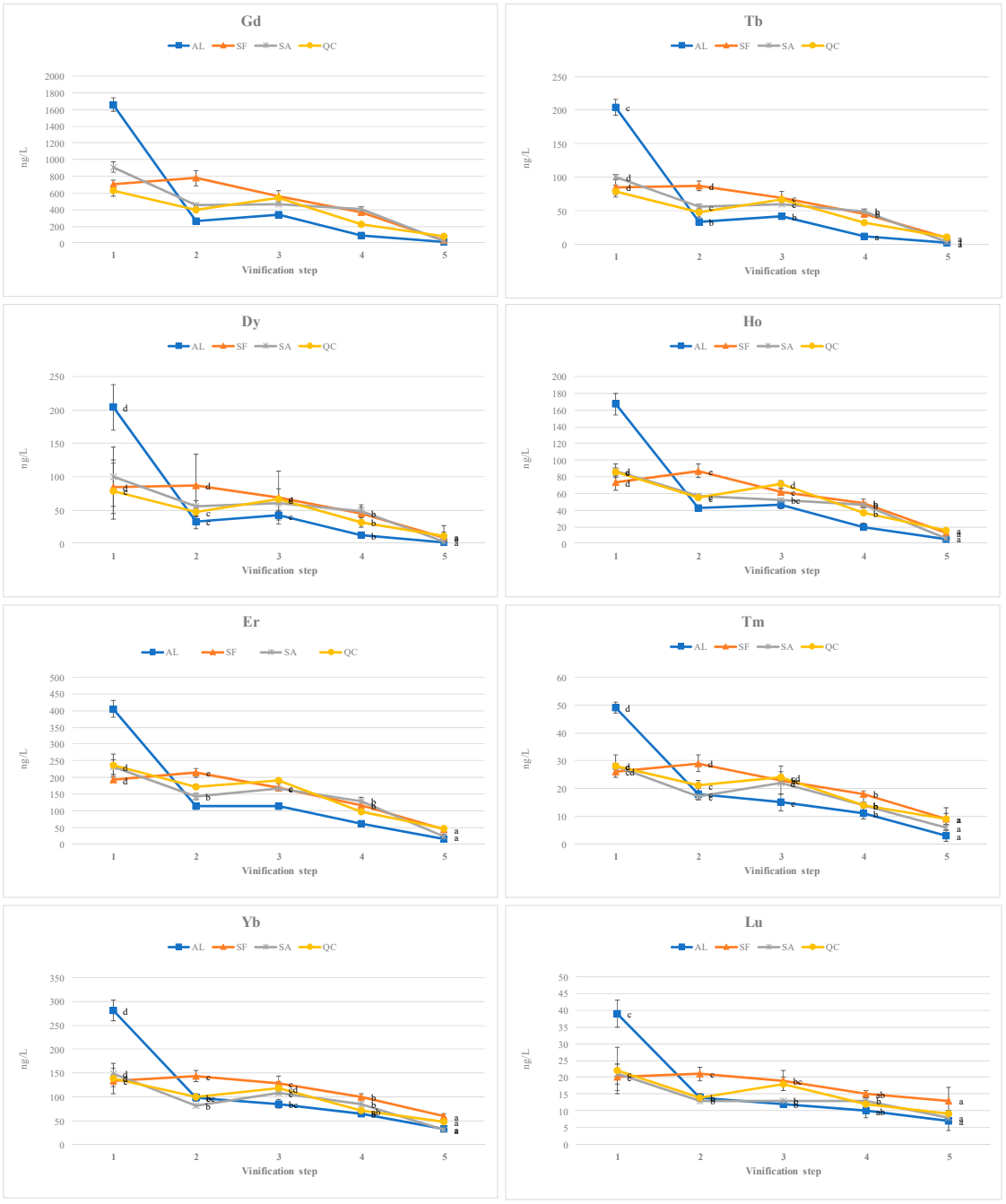

Figure 6. Evolution of rare earth elements concentrations over vinification, by vineyard of origin. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (QC) Quinta dos Carvalhais. Vinification step: (1) after crushing and vatting; (2) after must corrections and inoculation; (3) during the alcoholic fermentation (density approximately of 1030 g/L); (4) after running off; (5) after malolactic fermentation and first racking, before any blending to preserve the trace to the vineyard of origin. For each element and vineyard, results correspond to mean values (and corresponding standard deviations) of three replicates and corresponding analytical duplicates (n = 6). Means followed by the same letter are not significantly different at 0.05 level of significance. Values not followed by significance letters indicate that a non-parametric parametric test (Kruskal-Wallis test) was applied and comparison of means was not carried out.

3.3. Mineral Composition of Wines

The mineral composition of wines is of relevant interest due to its influence on physical-chemical stability, sensory characteristics, wine safety, legal limits, and as a discriminating tool for classification [2,10].

For most of the elements, significant differences were observed between wines from different DOs (Table 3). The wine from Dão DO showed significantly higher concentrations of Li, Be, Rb, Sn, Cs, and REE than the wines from the other DOs, which is consistent with elemental contents of soils.

Table 3. Multi-elemental composition of wines (2009 vintage) from the different vineyards. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (CQ) Quinta dos Carvalhais.

Element	Vineyard Effect	AL Vineyard/Palmela	SF Vineyard/Óbidos	SA Vineyard/Óbidos	QC Vineyard/Dão
Li	**	3049 ± 345 a	6323 ± 241 b	8084 ± 230 c	10,550 ± 1017 d
Be	*	251 ± 7	358 ± 17	222 ± 18	539 ± 139
Na	**	14,633 ± 639 c	15,640 ± 1166 c	10,706 ± 200 b	6480 ± 724 a
Mg	**	91,990 ± 5101 b	100,454 ± 6075 b	76,962 ± 1268 a	99,038 ± 10,380 b
Al	**	597 ± 51 c	198 ± 25 b	112 ± 6 a	129 ± 18 a
Ca	**	45,368 ± 3069 ab	56,819 ± 4786 c	41,245 ± 472 a	50,980 ± 4935 bc
Cr	*	24,997 ± 3286	18,189 ± 120	114,734 ± 1530	20,166 ± 2344
Mn	**	407 ± 21 a	1356 ± 94 d	1184 ± 14 c	1008 ± 98 b
Fe	*	1043 ± 65	1689 ± 111	1095 ± 8	186 ± 30
Ni	**	11,841 ± 1442 b	16,100 ± 3393 c	9177 ± 1085 ab	6838 ± 1563 a
Cu	*	16 ± 1	78 ± 3	92 ± 1	78 ± 16
Zn	**	940 ± 17 d	414 ± 33 c	259 ± 24 b	320 ± 32 a
Ga	*	841 ± 9	704 ± 16	984 ± 14	870 ± 100
Rb	*	4037 ± 320	2694 ± 209	2638 ± 46	7228 ± 761
Sr	**	309 ± 18 b	322 ± 22 b	222 ± 9 a	219 ± 24 a
Mo	**	1039 ± 75 b	317 ± 45 a	329 ± 80 a	1073 ± 73 b
Sn	*	137 ± 39	205 ± 27	934 ± 336	1032 ± 43
Sb	**	442 ± 54 b	250 ± 32 a	410 ± 74 b	161 ± 48 a
Cs	*	3543 ± 294	2344 ± 158	2686 ± 15	64,716 ± 6609
Ba	**	194 ± 23 bc	222 ± 21 c	167 ± 4 ab	138 ± 26 a
La	*	209 ± 79	193 ± 18	186 ± 9	634 ± 84
Ce	*	177 ± 144 a	207 ± 20 ab	123 ± 26 a	347 ± 73 b
Pr	**	20 ± 14 a	40 ± 8 b	31 ± 6 ab	85 ± 10 c
Nd	*	58 ± 57	161 ± 5	111 ± 9	347 ± 65
Sm	**	7 ± 9 a	26 ± 4 b	12 ± 6 a	46 ± 6 c
Eu	*	40 ± 6 a	50 ± 2 b	49 ± 2 ab	44 ± 8 ab
Gd	**	16 ± 12 a	34 ± 5 a	23 ± 3 a	85 ± 14 b
Tb	**	2 ± 3 a	9 ± 3 b	3 ± 1 a	11 ± 1 b
Dy	**	17 ± 15 a	43 ± 3 b	20 ± 3 a	65 ± 16 b
Ho	**	5 ± 2 a	13 ± 1 b	6 ± 2 a	16 ± 0 c
Er	**	16 ± 4 a	44 ± 7 b	23 ± 6 a	47 ± 2 b
Tm	*	3 ± 2 a	9 ± 4 b	6 ± 1 ab	9 ± 2 b
Yb	**	33 ± 4 a	59 ± 5 c	31 ± 3 a	47 ± 7 b
Lu	*	7 ± 3 a	13 ± 4 b	8 ± 1 a	9 ± 1 a
W	*	2 ± 1 ab	0.3 ± 0.2 a	1 ± 1 a	3 ± 2 b
Tl	*	1174 ± 56	206 ± 14	659 ± 6	1173 ± 109

* Significant effect ($p < 0.05$); ** significant effect ($p < 0.01$). For each vineyard and element, the average of the three replicates (considering the final winemaking step: after alcoholic fermentation and first racking) was calculated. The results are expressed as ng/L, with exception of Na, Mg, Al, Ca, Mn, Fe, Cu, Zn, Rb, Sr, and Ba, expressed as µg/L. Means followed by the same letter are not different at $p < 0.05$.

Wines from Óbidos vineyards showed similar concentrations of Rb and Cs, reflecting the soil composition. Despite the apparent higher transference of mineral elements from soil to grapes and must from Palmela vineyard, the resulting wine tends to present lower concentrations in comparison with the wines from the other vineyards. A reasonable explanation for these results can be the more intense precipitation through time favoured by the wine physical-chemical composition.

In comparison with other Portuguese DOs, wine Li concentrations in the study DOs were lower than those reported for the Douro DO (about 30 µg/L), where soils are developed on schists [4], while Mg and Mn concentrations were lower than those shown by Alentejo DO wines (close to 140 mg/L and 3 mg/L, respectively; [21]) from vineyards grown in soils rich in Mg [41]. Also, the concentrations of Li, Mg, Ca, Mn, Rb, Sr, and Cs were much higher than those reported for wines reflecting the complex of soil types in the Stellenbosch wine region [8]: 0.12–0.24 µg/L (Li), 9711–14,024 µg/L (Mg), 3789–10,343 µg/L (Ca), 94–228 µg/L (Mn), 199–630 µg/L (Rb), 41–102 µg/L

(Sr) and 0.30–6.71 µg/L (Cs). The Be and Rb may be useful to discriminate Portuguese from Romanian wines as the concentrations reported were, respectively, much higher and much lower [22] than those measured in the present study. Moreover, Rb concentrations are higher compared with those of German wines (160 to 970 µg/L), from Baden, Rheingau, Rheinhessen, and Pfalz wine regions [5].

Concentrations of Cu and Zn were very low in all the wines, and much lower than the OIV maximum acceptable limits of 1 and 5 mg/L respectively [45]. In fact, for all elements, concentrations are in accordance with the respective normal variation range, considering the grape variety and winemaking technology [2,10].

For those elements where a significant effect of Vineyard was found, PCA was performed, the results being displayed in Figure 7. The projection of the wines concerning the two vintages (2009 and 2010) in the C1-C2 plane was consistent with the projection of soils. The first two principal components, generated from PCA analysis of the data, explained 69.5% of the total variance. The variables which influence most the first component (C1, 47.5%) were Mn and REE, and the variables which influence most the second component (C2, 22.0%) Li, Be, Rb, and Cs. The projection of the wines in the C1-C2 plane presents some similarities with the projection of soils (Figure 3).

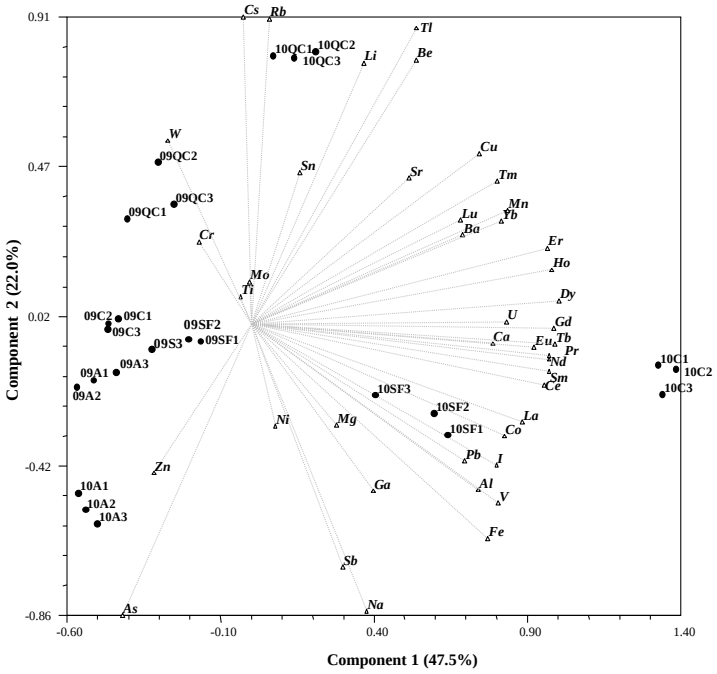

Figure 7. Principal Component Analysis performed on elemental wine composition (2009 and 2010 vintages) from the vineyards/DO: (AL) Vinha de Algeruz/Palmela, (SF) Quinta de S. Francisco/Óbidos, (SA) Quinta do Sanguinhal/Óbidos, and (QC) Quinta dos Carvalhais/Dão, collected at the final step of the vinification processes (5), in triplicate (1, 2, 3). Wines are represented in the plane of the two first components which express, respectively, 47.5% and 22.0% of the total variance.

3.4. Vineyards and Vintage Year

Results concerning the two vintages, 2009 (Table 3) and 2010 (Table S4) suggest the influence of the vintage year on wine elemental composition, as different hydrological conditions occurred during the study period; for instance, the annual rainfall in 2009 (especially during spring) was lower than the climatological normal, whereas in 2010 was much higher. Yet, with regards to DA results, considering both the wines from 2009 and 2010 vintages (six samples from each vineyard), Li, Mn, Sr, and REE allowed wine discrimination according to vineyard/geographical origin, suggesting their robustness to vintage effect (Figure 8). These results agree with findings of a study involving Australian wines from 19 vintages, which indicated that the multi-element composition is essentially independent of

their vintage [7]. Also, they follow the trend observed for REE in wines from different vintages and from the same vineyard over a period of several years [44].

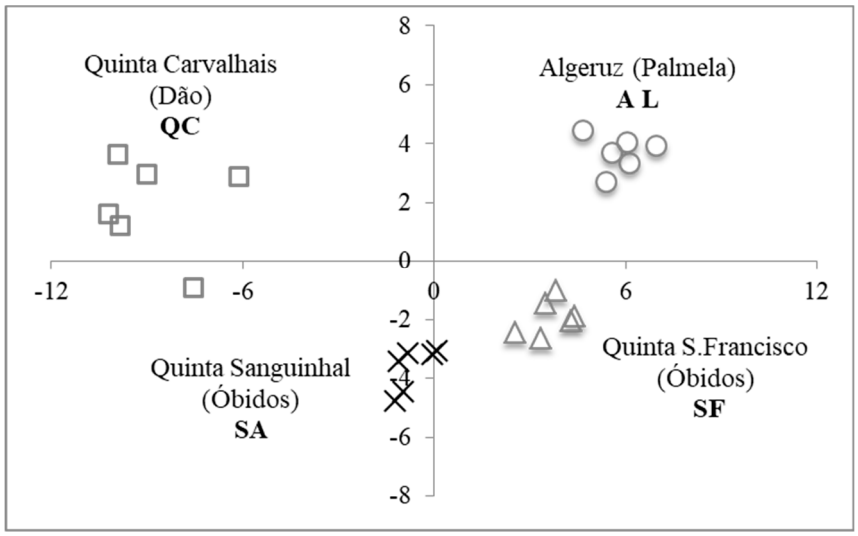

Figure 8. Scores plot of discriminant analysis (DA) of wine samples (2009 and 2010 vintages, vinification step 5) from different vineyards/DO, using Li, Mn, Rb, Sr and REE as variables.

Despite the controversy associated with their potential contamination [11,46], it is worthwhile to emphasize the role played by REE as discriminant elements [6,20]. This role can be better explored by the chondrite-normalisation approach, which allowed strong correlation to be observed between the REE patterns of grape musts and those of the provenance soils from Portuguese DOs [6]. Also, the REE patterns of wines from different vineyards in France, California, and Australia revealed inter-regional variations [44].

Despite the elemental concentration variations observed throughout vinification, the elemental composition of the wines reflected the elemental composition of the provenance soils for some elements. Differences were observed between the multi-elemental compositions of wines from different Portuguese vineyards/geographical origins, which enabled their successful discrimination through the application of multivariate statistics. Furthermore, the potential applicability of this strategy for intra-regional classification of wines was demonstrated. The results of this study obtained by application of a geochemical approach represent a valuable contribution both for viticultural zoning and for the building a database concerning Portuguese wines. Nevertheless, further research should be performed involving all the representative lithological formations of the studied DOs in order to characterize each region, as well as other Portuguese DOs. Moreover, the influence of the vintage year, in direct relationship especially with climate changes, requires further research.

Supplementary Materials: The following are available online at http://www.mdpi.com/2306-5710/4/4/85/s1, Table S1: Mean (±1 SD, n = 9) values of coarse fragments >2 mm (CF), sand (SD), silt (SI), clay (CL), pH, organic carbon (orgC), base cations and sum of bases (SB), phosphorus by Olsen test (P_{OL}), iron and aluminium determined by the dithionite-sodium citrate method in the samples from the vineyards of Algezuz (AL), Quinta de São Francisco (SF), Quinta do Sanguinhal (SA), and Quinta de Carvalhais (QC) at 0–20, 40–60 and 60–80 cm depth; Table S2: Effect of depth level on soil multi-element composition (µg/g): (a) Algeruz (AL) vineyard; (b) Quinta de S. Francisco (SF) vineyard; (c) Quinta do Sanguinhal (SA) vineyard; (d) Quinta dos Carvalhais (QC) vineyard; Table S3: Vineyard effect on multi-elemental composition of grape berries (corresponding musts) collected in the vineyards of Algezuz (AL), Quinta de São Francisco (SF), Quinta do Sanguinhal (SA), and Quinta de Carvalhais (QC); Table S4: Multi-elemental composition of wines (2010 vintage) from the different vineyards. (AL) Vinha de Algeruz; (SF) Quinta de S. Francisco; (SA) Quinta do Sanguinhal; (CQ) Quinta dos Carvalhais.

Author Contributions: S.C., A.C.-G., M.M. and R.B.d.S. conceived and designed the experiments; S.C. and F.M. performed the experiments; S.C., M.M. and I.C. analyzed the data; S.C., A.C.-G., M.M. and R.B.d.S. contributed reagents/materials/analysis tools; S.C. and M.M. wrote the original draft; S.C., M.M., I.C., A.C.-G. and R.B.d.S. revised the paper.

Funding: This research was supported by the Portuguese National Funding Agency for Science and Technology through the R&D project PTDC/AGR-ALI/64655/2006, grant SFRH/BPD/93535/2013, and through the research centre LEAF (UID/AGR/04129/2013).

Acknowledgments: The authors acknowledge the enterprises Companhia Agrícola do Sanguinhal Lda, José Maria da Fonseca Vinhos, and Sogrape Vinhos for providing their facilities regarding the project development; Paulo Marques and José Correia for help in field sampling; the staff of the Soil Laboratory (ISA, Lisbon) for soil analysis; and Otília Cerveira for help in Mineral Analysis Laboratory activities (INIAV, Dois Portos).

Conflicts of Interest: The authors declare no conflict of interest.

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